

What lays beneath a neglected ocular trauma in a child?

Anca Delia Pantalon¹, Irina Andreea Pavel^{2*}, Roxana Popescu³, Delia Ciobanu⁴, Dorin Chiseliță^{1,2}, Camelia Margareta Bogdănici^{1,2}

¹ "St. Spiridon" Emergency University Hospital - Ophthalmology Clinic, Iași, Romania

² "Grigore T. Popa" University of Medicine and Pharmacy – Ophthalmology Department, Iași

³ "Grigore T. Popa" University of Medicine and Pharmacy – Clinical Genetics and Immunology Department

⁴ "Grigore T. Popa" University of Medicine and Pharmacy – Pathology Department, Iași

Abstract. Aim of study: The diagnosis of leukocoria may be missed especially in a context of ocular trauma. We report the case of a 3 years old boy, incidentally diagnosed with bilateral retinoblastoma after a neglected penetrating globe injury caused by a sharp wooden object in OD. **Material and method:** Initial clinical evaluation detected in OD marked conjunctival congestion, "cork-screw" dilated episcleral vessels, buphthalmos with enlarged corneal diameter ($\varnothing=18$ mm), corneal edema, 2mm hyphema, iris neovascularization with active bleeding, shallow anterior chamber (AC), non-reactive mydriatic pupil, partially opacified lens and increased intraocular pressure (IOP=46 mmHg); in the infero-nasal quadrant the scleral wall seemed discontinuous, but underlying uvea and a large adjacent hematoma sealed the scleral wound. In OS clinical examination revealed severe uveitis signs: 360° posterior iris synechiae, a 3 mm hypopion, dense pupillary fibrinoid membrane and white cataract. Ultrasound examination (mode B) revealed massive vitreous hemorrhage and choroidal hematomas, more prominent in OD. Intensely hyper-reflective images, pleading for intraocular calcifications ($\varnothing=1-3$ mm) were identified in both eyes. Total retinal detachment (funnel shape) was visible in OS. Cerebral computer tomography (CT) scan confirmed bony density (≈ 1000 UH) of the intraocular hyperreflective images, highly indicative for bilateral multiloculated retinoblastoma; extraocular extensions towards the anterior orbit and optic nerve were described in OD. Extended enucleation with long optic nerve stump was completed in OD; pathology described extremely aggressive (Ki67=58%), anaplastic retinoblastoma (pT4Nx – G3L1V1Pn1) with both endo- and exophytic growth pattern, involving anterior intraocular structures, orbital fat tissue and adjacent striated muscular fibers, optic nerve and lamina cribrosa. Karyotype analysis was normal. After discharge, patient was referred to Pediatric Oncology for further adjuvant treatment, but rapid systemic spread was noted with extreme alteration of life quality and guarded survival rate, despite all efforts. **Conclusion:** Neglected initial diagnosis, delayed presentation dictated by ocular trauma prevented proper timely management and better survival rate in such case.

Key words: neglected ocular trauma, bilateral retinoblastoma, primary orbital retinoblastoma

INTRODUCTION

Ocular trauma represents almost 10% of all reported injuries in children, being the most common cause of unilateral blindness in pediatric age (1). Severe ocular trauma has been estimated at 8.85 to 15.2 cases per 100.000 (2). Clinical presentation is highly diverse, from minor subconjunctival hemorrhage to traumatic cataracts, hemophthalmos, traumatic retinal detachment etc. Usually, accurate diagnosis can be challenging due to non-compliance at

Corresponding author: irinaandreea.pavel@gmail.com

Received: June 30, 2023; **Accepted:** October 1, 2023;

Published: December 30, 2023

Citation: Pantalon Anca Delia, Pavel Irina Andreea, Popescu Roxana, Ciobanu Delia, Chiseliță D., Bogdănici Camelia Margareta: What lays beneath a neglected ocular trauma in a child. Journal of Surgery [Jurnalul de chirurgie]. 2023; 19(4): 321 - 329, DOI: 10.22551/JSURG.2023.04.08.

Copyright © 2023: Pantalon Anca Delia. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

examinations or lack of timely presentation. Immediate adequate treatment is highly dependable on these factors.

In children the incidence of retinoblastoma has been described between 1:15000 to 1:20.000 live births (3). Main cause is represented by biallelic inactivation of RB1 gene located at 13q14.2 (4, 5). The most frequent form is the non-hereditary type of tumor (60%), with both inactivating events occurring at the level of the retinal cells directly. In these circumstances, the tumor develops unilaterally in the first three years of life. For the remaining situations (40%), the genetic mechanism in the hereditary tumors involves a germ line mutation ("first hit" event) and a subsequent inactivation of the other allele "second hit" event) necessary for the tumor to form (6, 7). The tumors are generally multifocal, bilateral or even trilateral, if associated with intracranial primitive neuroectodermal tumors; the age of diagnosis is below 12 months of age.

Hereditary retinoblastoma is transmitted autosomal dominantly with incomplete penetrance, in almost 90% cases (7). Few subjects remain unaffected or develop benign lesions, called retinomas (8). Primary orbital retinoblastoma (PORb) can be evoked and represents a fatal disease (9). In developed countries, the incidence of PORb has been reported between 6.3-7.6%, yet in developing countries this varies between 18% and 36-40%. Poor education, delayed presentation and limited access to healthcare contribute to high mortality rate at 5 years (50%-90%), (10, 11), compared to highly industrialized countries, where survival reaches 88%-90% at 5 years (12-15). As such early detection and genetic screening represent key points in improving prognosis and survival rate of retinoblastoma diagnosis.

Clinical context

In February 2018 the patient P.D. male, 3 years old, rural area is referred to our Ophthalmology Clinic for a neglected ocular trauma (wooden stool from household) in OD (oculus dexter), occurred 2 weeks before the current presentation. Previous ophthalmological examination from April 2017 in a non-tertiary

center indicated esotropia and bilateral leucocoria due to white cataracts; recommendation at that point was to address a tertiary center for further investigations and specialized treatment.

Patient's personal history showed nothing notable in his early childhood, all five older siblings were healthy (declaratively). Family history revealed in the father a positive diagnosis for an intraocular tumor, reason why his OD was enucleated. No medical records or pathology results could be presented to support a specific diagnosis. Written informed consent was obtained from the parents before the investigations and surgical procedure were started.

Clinical examination recorded an altered general status with low weight/height for age ratio. Ophthalmological examination under sedation with sevoflurane detected in OD globe dystopia towards the inferotemporal quadrant (Fig. 1), marked conjunctival congestion, "cork-screw" dilated episcleral vessels, buphthalmos with enlarged corneal diameter ($\varnothing=18$ mm), corneal edema, hyphema, iris neovascularization, shallow anterior chamber (AC), non-reactive mydriatic pupil, partially opacified lens and secondary neovascular glaucoma with IOP OD=46 mmHg; active bleeding in the pupillary area with a potential source from the retro-iris space, subluxated lens.



Fig. 1. General anesthesia examination: OD extremely thin sclera/360°, neovascular glaucoma with active bleeding from the iris neovascularization, secondary exotropia. OS severe uveitis with hypopyon.

In the infero-nasal quadrant the scleral wall seemed ruptured, but a large mass apparently consisting in uvea and a large hematoma sealed the scleral wall post-traumatic defect (Fig. 2 a,b).

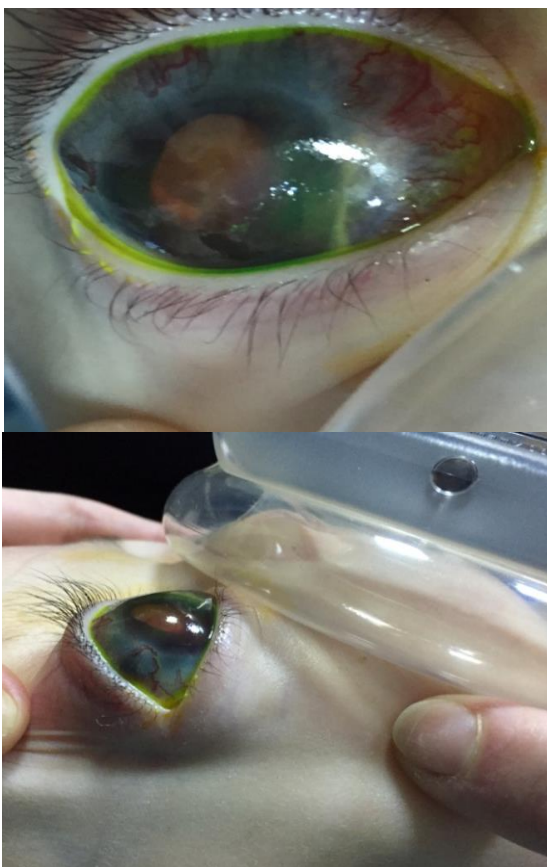


Fig. 2a, b OD (detail) Discontinue scleral wall in the infero-nasal quadrant sealed by a subconjunctival mass, adjacent to a corneal erosion area; positive fluorescein test showing epithelial defect ($\approx 1/3$ corneal surface);

In OS (oculus sinister) clinical examination revealed severe uveitis signs: 360° posterior iris synechiae, a 3 mm shifting hypopion, dense fibrinoid membrane in the pupillary area and white cataract (Fig. 3). Corneal diameter in OS was $\varnothing=13.5$ mm and IOP OS=20 mmHg.

Ultrasound examination (mode A&B) revealed massive vitreous hemorrhage, large choroidal and retrobulbar hematomas in OD. Intensely hyper-reflective images ($\varnothing=1-3$ mm) were identified in both eyes, pleading for intraocular calcifications. Total retinal detachment was visible in OS.



Fig. 3 OS (detail) severe uveitis with shifting 3mm hypopion, complicated white cataract;

Orbit and brains computer tomography (CT) scans in axial and coronal orientation with 2mm slice thickness detected the bony density (≈ 1000 UH) of the intraocular hyper-reflective images in both eyes, suggestive for bilateral retinoblastoma (Fig. 4a, b); enlarged right globe with irregular mass occupying the anterior orbit and optic nerve area were aspects consistent with primary orbital retinoblastoma in OD. In OS there was identified a single site tumor, with no extra-scleral extension. Both bony orbits were radiologically normal. No other cerebral changes could be identified at this point.

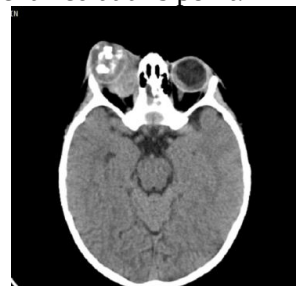


Fig. 4a. Cerebral CT scan. Slice showing in OD primary orbital retinoblastoma (orbital extension of an intraocular retinoblastoma at initial presentation, manifesting as proptosis and buphthalmos due to neovascular glaucoma); disseminated intraocular calcifications suggest multilocular retinoblastoma;

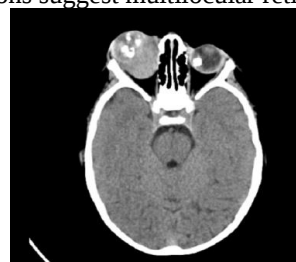


Fig. 4b. Cerebral CT scan. Slice showing a single site intraocular calcification, without scleral extension in OS

Further examinations, including palpation of the regional lymph nodes, chest X-ray, abdominal ultrasonography, blood/urine tests and CSF cytology aimed to detect any potential metastatic spread. No systemic metastatic spread was detected and most parameters were within normal limits, except some that pointed out mild lymphocytosis, low hemoglobin levels and erythrocytic line changes indicating moderate-to-severe microcytic anemia. Neo-adjuvant chemotherapy for tumor reduction could not be recommended in this case due to the open globe trauma with massive local hematoma at the site. In these circumstances, enlarged modified enucleation was performed with long optic nerve stump; under direct visualization after lateral canthotomy, orbital tumor extension was completely (macroscopically) removed, with utmost care for tissue manipulation during the procedure. Immediate blood aspiration and meticulous hemostasis to limit tumor cell seeding was performed, yet the ruptured scleral wall due to recent globe trauma was difficult to manage even for an experienced surgeon.

Histology described in the enucleated eye focal invasion of the ciliary body (Fig. 5a), iris, anterior chamber, choroid >3mm, sclera, episclera, orbital fat tissue and adjacent striated muscular fibers from the extraocular muscles. Intraocular calcifications and retinal detachment were confirmed (Fig. 5b), optic nerve (anterior segment of the stump) and lamina cribrosa were also infiltrated (Fig. 5c).

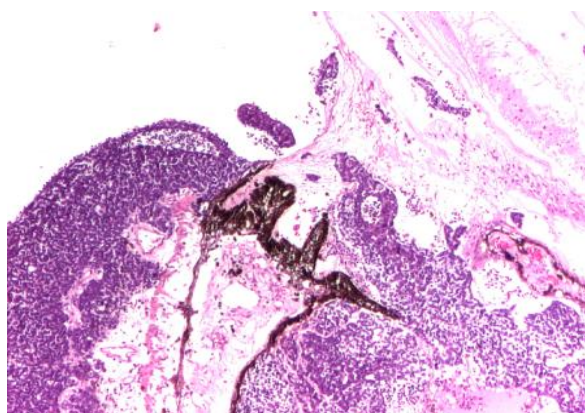


Fig. 5a Extension into the ciliary processes; HE staining x4 magnification

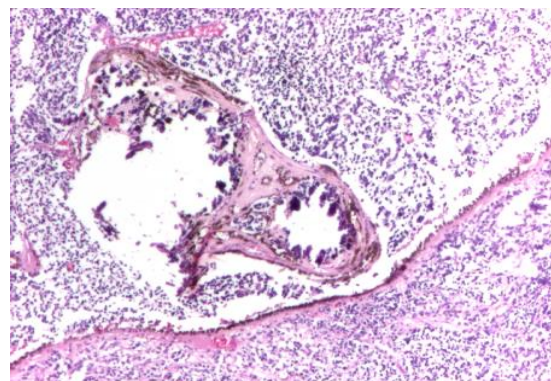


Fig. 5b Stromal calcifications and retinal detachment; HE staining x4 magnification

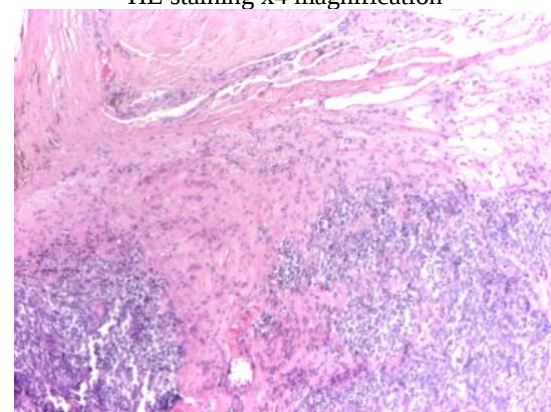


Fig. 5c Optic nerve extension in the resection stump; HE staining x4 magnification

Tumor grading revealed undifferentiated retinoblastoma (pT4Nx – G3L1V1Pn1) with both endo- and exophytic growth pattern; total retinal detachment accompanied massive tumor (~90% vitreous cavity, 2.3/2.2.2.3cm); anaplastic cells (reduced cytoplasm and large, hyperchromic, irregular, angulated nuclei) were identified, majority without a true rosette arrangement (pseudo-rosette) (Fig. 6a); few Flexner-Wintersteiner and Homer-Wright rosettes were sporadically identified, (Fig. 6b).

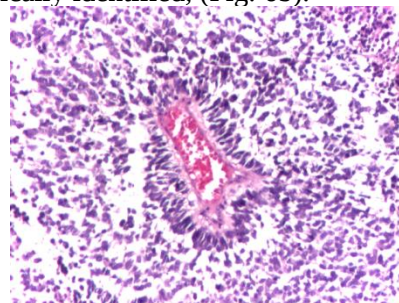


Fig. 6a Pseudo-rosette (detail), HE staining, 10x magnification

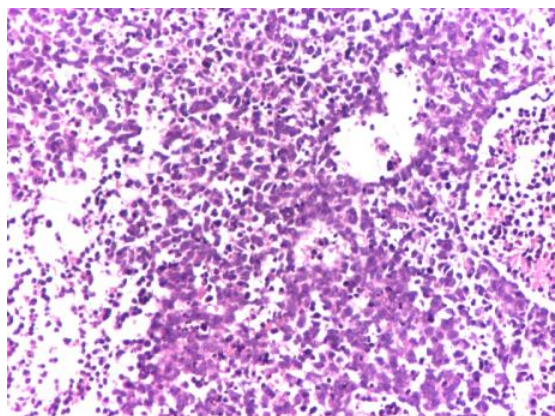


Fig. 6b Flexner-Wintersteiner and Homer-Wright rosettes; HE staining, 10x magnification

Frequent, atypical mitosis were encountered (approx. 12/10HPF, high-power field) together with both lymphatic/blood vessels and perineural invasion. Wide areas of necrosis/calcifications and reduced fibrovascular stroma could be described (Fig. 6c).

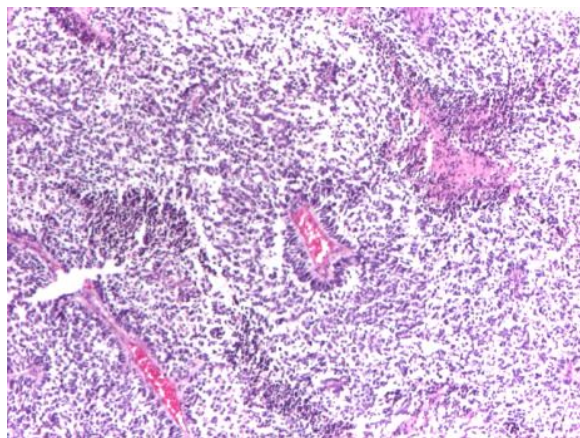


Fig. 6c Perivascular invasion and necrotic areas; HE staining, 4x magnification

Immunohistochemistry (IHC) staining showed cytoplasmic positivity for synaptophysin and neuron-specific enolase (NSE) in the tumor cells, Fig. (7a, b); staining for S100 was inconclusive and Ki67 antigen, describing the intratumoral variation in mitotic activity, was 58% (Fig. 7c).

Karyotype analysis and analysis of the copy number variation in exons 6, 14, 19, 24, 26 of *RB* gene by MLPA (Multiplex Ligation-Dependent Probe Amplification) with P335

probe mix revealed no abnormalities in our patient.

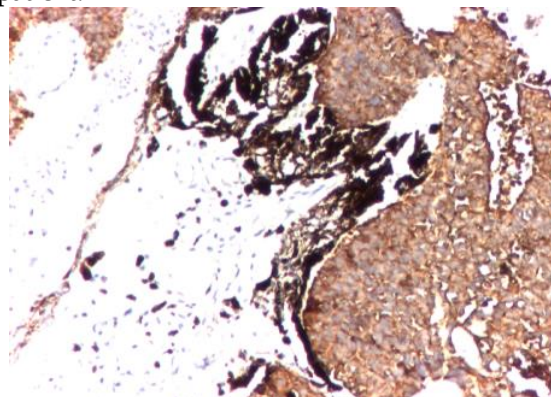


Fig. 7a IHC staining, Synaptophysin (+), 20x magnification (detail)

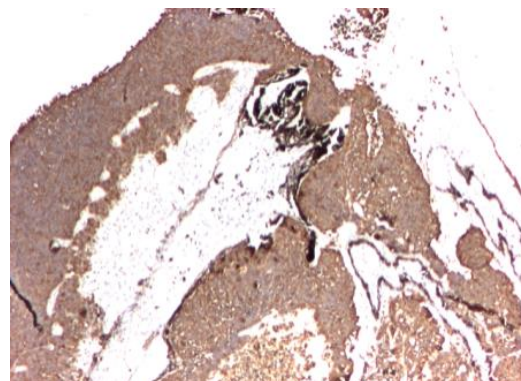


Fig. 7b IHC staining, neuron-specific enolase (NSE) diffuse spread, 4x magnification

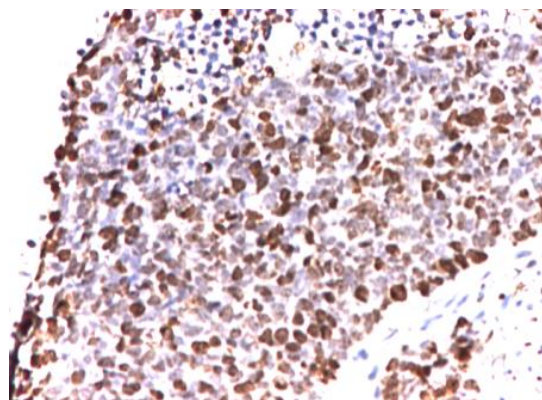


Fig. 7c IHC staining, Ki67 antigen, 20x magnification (detail)

At discharge from our Ophthalmology service, the patient was referred to a Pediatric Oncology Clinic for further treatment (systemic chemotherapy and external beam radiotherapy).

Triple regimen intravenous chemotherapy (IVC) protocol was initiated in high-doses (day 1: Carboplatin 28mg/kg body weight, Etoposide 6mg/kg body weight and Vincristine 0.025mg/kg body weight; day 2: Etoposide 6mg/kg body weight); negative impact upon general status required IVC cessation after 2 sessions (3 weeks apart), due to increased IVC-related toxicity (severe refractory anemia, neutropenia and gastrointestinal toxicity). As such rapid progressive disease appeared (Fig. 8).



Fig. 8 Orbital recurrence with massive tumor growth despite systemic high-dose chemotherapy (7 weeks post-op, April 2018)

Supportive palliative care was initiated as well as continuous hospital monitoring (Fig. 9).



Fig. 9 Advanced local and systemic metastatic disease - palliative care, Pediatric Oncology Clinic (4 months post-op, June 2018);

Still, despite all efforts, in July 2018 exitus occurred at 4 months postoperative due to systemic advanced metastatic disease (brains, liver, bones, lungs).

DISCUSSION

Ocular trauma, especially in young children could rarely mask/ coincide with other ocular pathologies, therefore clinicians should be highly aware of different potential diagnoses in such cases; comprehensive examination should always be performed before anything else. Retinoblastoma is the most frequent malignant tumor of neuroectodermal origin (4%) in children (16), which appears as a solid intraocular tumor, typically before the age of 5 years (17). Leukocoria represents the most common sign of retinoblastoma. Nevertheless, retinoblastoma may also present with unusual signs, such as vitreous hemorrhage or inflammation, leading to misdiagnosis and inappropriate treatment. Common symptoms related to ocular trauma may mask the signs of retinoblastoma (18). In rare cases, spontaneous globe rupture secondary to retinoblastoma can develop (19). If bilateral (35-40% in all cases) or multilocular at presentation hereditary transmission should always be suspected and investigated (20). When tumor is located strictly intraocular at presentation, enucleation combined with other neo-/adjuvant treatments has a cure rate of rate of 90-95% (16, 21). Orbital recurrence (secondary orbital retinoblastoma) after enucleation for primary confined intraocular tumors is described in 4.2-9.5% cases (22, 23). Primary orbital retinoblastoma (detected either clinically or radiologically at first presentation, as it was the case in our patient) includes a wide range of clinical entities, varying with the tumor load; it is rare as first presentation nowadays in developed countries (6.3-7.6%) (10, 11), but in developing countries it still remains one of the major forms (9). Survival rates at 5 years in orbital retinoblastoma are remarkable in countries like UK or Japan, whereas in developing countries mortality is as high as 50-90%. In these cases, if multimodal treatment is applied, survival rates improve considerably, on condition that treatment is started as soon as possible (9). A detailed and comprehensive visual assessment should be performed for all retinoblastoma survivors. Decreased contrast sensitivity and impaired saccades have been observed in

retinoblastoma survivors (24). In our case we encountered multiple negative aspects that rendered guarded prognosis since initial visit: delayed presentation, lack of genetic counseling and early testing for all off-springs, concomitant neglected open globe trauma and consecutive difficulties in intraoperative hemostasis, impossibility of neo-adjuvant chemotherapy for tumor reduction due to recent local injury complicated with active bleeding and voluminous hematoma.

Genetic testing by karyotyping revealed no abnormalities in this case. Nevertheless, the karyotype analysis has limited importance, mostly when microscopic deletions are present (25). Tumor development in bilateral familial retinoblastoma, as suspected in our case, is linked to biallelic suppression of the RB1 gene (26). Most forms of heritable retinoblastomas have a single mutation in the RB1 allele as the “first hit”, as it was supposedly the case in the father; Losing the second allele by other mechanisms (loss of heterozygosis, epigenetic dysregulation etc) is very likely to happen in fetal and infantile retinal cells, so that the chances in later development of at least one tumor could reach >90% in subjects inheriting initially a “first hit” mutation, as could be the case in the child presented in this case. Unaffected carriers are either subjects possessing an initial mutation but in whom, no sensitive retinal cell loses the remaining wild-type RB1 allele, or individuals who have an initial RB1 mutation with partial function (26, 27), as it was probably the case of the other siblings (only two were brought in for karyotyping assessment and clinical examination, with normal outcomes. Unfortunately, the father refused to give a blood sample for further investigations.

However, genetic transmission in this case relies upon anamnesis (unilateral tumor in a 7 months old child, with good survival at 30 years after enucleation) and the clinical presentation, as most heritable retinoblastomas are bilateral/multilocal. Genetic theories in similar cases advocate that the majority of RB1 mutations arise de novo, either pre- or post-conception (28). Pre-conception mutagenesis usually occurs during spermatogenesis. The affected child carries the de novo mutations in every cell, typically presenting

with 4-5 multilocal tumor and bilateral retinoblastoma, as it was the clinical presentation for our patient. Lack of an elaborated and exhaustive complete genetic analysis in all siblings amputates greatly the results and interpretation of the genetic transmission pattern in this family (28, 29).

Primary goal in retinoblastoma treatment is to save the patient's life; vision preservation and globe sparing strategies come only as secondary goals (30). Therefore, a high-dose protocol of intravenous chemotherapy (IVC) was considered, taking into account pros and cons for our decision in respect to life saving strategies (no systemic metastasis present, bilateral disease, orbital spread in OD, impossibility of neo-adjuvant chemoreduction, increased risk of dissemination during surgical manipulation of the opened globe, aggressive anaplastic tumor). Recent ICRB recommendations from November 2020 plead for high dose IVC if either bilateral disease or if choroid extension >2mm, extraocular extension or optic nerve extension are present (31). Despite the only 5-10% chances for developing toxicity (32), in our patient chemotherapy had to be ceased after 2 sessions only, for refractory neutropenia and gastrointestinal IVC-related toxicity, amplifying the very poor prognosis in this case.

However, in our case, the major cause for the poor survival and the guarded prognosis remained the delayed and masked (by trauma) diagnosis with advanced bilateral disease at presentation, combined with the low socio-economic status and poor compliance of the family. For the fellow ophthalmologists, this case should be an alert sign, as early detection (bilateral leucokoria) and genetic screening programs (genetic transmission in high suspicion) could increase the chances for survival in such cases.

CONCLUSIONS

Neglected initial diagnosis (bilateral leucokoria), delayed presentation hastened only by the context of ocular trauma prevented proper timely management and better survival rate in such case.

Each retinoblastoma case exhibits its own particularities. Variable features at

presentation could imitate other clinical entities, therefore the clinician should remain alert and aware of potential ocular pathologies; still the outcome in some cases is conditioned by early detection, available logistics and patient's education.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding the scientific research.

REFERENCES

1. Takvam JA, Midelfart A. Survey of eye injuries in Norwegian children. *Acta Ophthalmol (Copenh)* 1993;71(4):500-505.
2. Brophy M, Sinclair SA, Hostetler SG, Xiang H. Pediatric eye injury-related hospitalizations in the United States. *Pediatrics* 2006;117(6): e1263-e1271.
3. Broaddus E, Topham A, Singh AD. Incidence of retinoblastoma in the USA: 1975-2004. *Br J Ophthalmol* 2009;93(1):21-3.
4. Knudson AG Jr. Mutation and Cancer: Statistical Study of Retinoblastoma. *Proc. Nat. Acad. Sci.* 1971; 68(4):820-823.
5. Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, Dryja TP. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* 1986;323(6089):643-6.
6. Ariani F, Pinto AM, Renieri A. Retinoblastoma (hereditary predisposition). *Atlas Genet. Cytogenet Oncol Haematol* 2020; 24(2):97-101.
7. Valverde JR, Alonso J, Palacios I, Pestaña A. RB1 gene mutation up-date, a meta-analysis based on 932 reported mutations available in a searchable database. *BMC Genet* 2005;4(6):53.
8. Harbour JW. Molecular basis of low-penetrance retinoblastoma. *Arch Ophthalmol* 2001; 119(11): 1699-704.
9. Honavar SG, Manjandavida FP, Reddy VAP. Orbital retinoblastoma: An update. *Indian J Ophthalmol* 2017;65(6):435-442.
10. Singh AD, Shields CL, Shields JA. Prognostic factors in retinoblastoma. *J Pediatr Ophthalmol Strabismus* 2000; 37:134-41.
11. Ajaiyeoba IA, Akang EE, Campbell OB, Olurin IO, Aghadiuno PU. Retinoblastomas in Ibadan: Treatment and prognosis. *West Afr J Med* 1993; 12:223-7.
12. Young JL, Smith MA, Roffers SD, Liff JM, Bunin GR. Retinoblastoma. In: Ries LA, Smith MA, Gurney GJ, editors. *Cancer Incidence and Survival among Children and Adolescents: United States SEER Program 1975-1995. NIH Publication No. 99-4649.* Bethesda, MD: National Cancer Institute, SEER Program; 1999.
13. Sanders BM, Draper GJ, Kingston JE. Retinoblastoma in Great Britain 1969-80: Incidence, treatment, and survival. *Br J Ophthalmol* 1988; 72:576-83.
14. Anonymous Survival rate and risk factors for patients with retinoblastoma in Japan. The Committee for the National Registry of Retinoblastoma. *Jpn J Ophthalmol* 1992; 36:121-31.
15. Abramson DH, Niksarli K, Ellsworth RM, Servodidio CA. Changing trends in the management of retinoblastoma: 1951-1965 vs.1966-1980. *J Pediatr Ophthalmol Strabismus* 1994; 31:32-7.
16. Rao AA, Naheedy JH, Chen JY, Robbins SL, Ramkumar HL. A clinical update and radiologic review of pediatric orbital and ocular tumors. *J Oncol.* 2013; 2013:975908.
17. DeYoung C, Barke MR, Shields CL. Cavitary Retinoblastoma Imaged on Magnetic Resonance Imaging. *J Pediatr Ophthalmol Strabismus* 2023;60(1):e1-e4.
18. Fang Y, Li Q, Zhang C, Zhao J. Incidental detection of retinoblastoma from accidental trauma in children. *BMC Ophthalmol.* 2023;23(1):81.

19. Ng H, Sloan B, Ng YSP, Chong CF. Spontaneous globe rupture in infant with retinoblastoma. *Pediatr Blood Cancer*. 2023 Aug;70(8):e30326.
20. Aerts I, Lumbroso-Le Rouic L, Gauthier-Villars M, Brisse H, Doz F, Desjardins L. Retinoblastoma. *Orphanet J Rare Dis*. 2006; 1:31.
21. Sherief ST, Asfaw G, Abateneh A, Takewe S, Fufa D, Wondale T, Takele T, Dimaras H. Incidence and geographic distribution of retinoblastoma in Ethiopia. *BMC Ophthalmol*. 2023;23(1):231.
22. Kim JW, Kathpalia V, Dunkel IJ, Wong RK, Riedel E, Abramson DH. Orbital recurrence of retinoblastoma following enucleation. *Br J Ophthalmol* 2009; 93(4):463-7.
23. Arif M, Islam Z. Retinoblastoma: postenucleation orbital recurrence. *Can J Ophthalmol* 2010; 45(6):606-9.
24. Reynolds M, Wise J, Wu T, Malone S, Al Badawi A, King A, Gordon M, Lueder G, Hayashi R. Characterization of vision in pediatric retinoblastoma survivors beyond visual acuity. *J AAPOS* 2023;27(4): 188.e1-188.e6.
25. Joseph B, Paul P, Elamparithi A, Roy J, Vidhya A, Shanmugam M, Kumaramanickavel G. Karyotyping in retinoblastoma-a statistical approach. *Asian Pacific journal of Cancer Prevention: PJCP* 2005; 64: 468-471.
26. Tomar S, Sethi R, Sundar G, Quah TC, Quah BL, Lai PS. Mutation spectrum of RB1 mutations in retinoblastoma cases from Singapore with implications for genetic management and counselling. *PLoS One* 2017;12(6):e0178776.
27. Imperatore V, Pinto AM, Gelli E, et al. Parent-of-origin effect of hypomorphic pathogenic variants and somatic mosaicism impact on phenotypic expression of retinoblastoma. *Eur J Hum Genet* 2018; 26(7):1026-1037.
28. Kanber D, Berulava T, Ammerpohl O, Mitter D, Richter J, Siebert R, Horsthemke B, Lohmann D, Buiting K. **The Human Retinoblastoma Gene Is Imprinted.** *PLOS Genetics* 2009; 5(12): e1000790.
29. Eloy P, Dehainault C, Sefta M, Aerts I, Doz F, et al. **A Parent-of-Origin Effect Impacts the Phenotype in Low Penetrance Retinoblastoma Families Segregating the c.1981C>T/p. Arg661Trp Mutation of RB1.** *PLOS Genetics* 2016; 12(2): e1005888.
30. Pandey AN. Retinoblastoma: An overview. *Saudi J Ophthalmol* 2014;28(4):310-315.
31. Ancona-Lezama D, Dalvin LA, Shields CL. Modern treatment of retinoblastoma: A 2020 review. *Indian J Ophthalmol* 2020;68(11):2356-2365.
32. Brennan RC, Qaddoumi I, Billups CA, Kaluzny T, Furman WL, Wilson MW. Patients with retinoblastoma and chromosome 13q deletions have increased chemotherapy-related toxicities. *Pediatr Blood Cancer* 2016; 63:1954–1958.